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Winter, Gregory

<120> Selection System

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<140> 09/710,444

<141> 2000-11-20

<150> GB 9810223.9

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<150> GB 9810228.8

<151> 1998-05-13

<150> PCT/GB99/01526

<151> 1999-05-13

<160> 80

<170> PatentIn version 3.1

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Pro Ala Gly Leu Ser Glu Gly Ser Thr Ile Glu Gly Arg Gly Ala His
Glu
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ggcaccetea gaacggtace ceacceteag aggeeggetg ggeegeeace eteagag 57
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ggtggcggcc cagccggcct ttctgagggg tcgactatag aaggacgagg gcccagcgaa
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ggaggtggg taccccttc tgagggtgg
                                                                    89
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<400> 4
ccaccctcag aagggggtac cccacctcct tcgctgggcc ctcgtccttc tatagtcgac
                                                                     60
                                                                     89
ccctcagaaa ggccggctgg gccgccacc
<210> 5
<211> 24
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<220>
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<223> Synthetic PCR primer for vector construction/screening
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gcgatggttg ttgtcattgt cggc
                                                                     24
<210> 6
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<223> Synthetic PCR primer for vector construction/screening

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aaaagaaacg caaagacacc acgg
                                                                     24
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<223> Synthetic PCR primer for vector construction/screening
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cctcctgagt acggtgatac acc
                                                                     23
<210> 8
<211> 24
<212> DNA
<213> Artificial sequence
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<220>
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<223> Synthetic PCR primer used to screen for recombinant clones
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<400> 6

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gtaaattcag agactgcgct ttcc
                                                                     24
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attttcggtc atagcccct tattag
                                                                     26
<210> 10
<211> 65
<212> DNA
<213> Artificial sequence
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<223> Synthetic PCR primer recognizing FLAG tag nucleotide sequence.
<220>
<221> misc_feature
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<223> Synthetic PCR primer recognizing FLAG tag nucleotide sequence

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caaacgggcg gccgcagact acaaggatga cgacgacaag gaaactgttg aaagttgttt
                                                                      60
agcaa
                                                                      65
<210> 11
<211> 51
<212> DNA
<213> Artificial sequence
<220>
      Synthetic PCR primer used to change codon usage in recombinant cl
<223>
       ones.
<220>
<221> misc_feature
<222> (1)..(51)
<223> Synthetic PCR primer used to change codon usage in recombinant cl
       ones
<400> 11
cccctcagaa aggccggctg ggccgccgcc agcattgaca ggaggttcag g
                                                                      51
<210> 12
<211> 52
<212> DNA
<213> Artificial sequence
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<223>
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<400> 10

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| <400> gaagga | 12 ggtg gggtacccgg ttccgagggt ggttccggtt ccggtgattt tg | 52 | | |
|--|---|----|--|--|
| <210> | 13 | | | |
| <211> | 36 | | | |
| <212> | DNA | | | |
| <213> | Artificial sequence | | | |
| | | | | |
| <220> | | | | |
| <223> | Synthetic PCR primer for vector construction/screening. | | | |
| <220> | | | | |
| <221> | misc_feature | | | |
| <222> | (1)(36) | | | |
| <223> | Synthetic PCR primer for vector construction/screening | | | |
| | | | | |
| <400> | | 36 | | |
| ccctcggaac cggtacccca gctgcttcgt gggccc 36 | | | | |
| <210> | 14 | | | |
| <211> | 47 | | | |
| <212> | DNA | | | |
| <213> | Bacillus amyloliquefaciens | | | |
| | | | | |
| <400> | 14 | 47 | | |
| ctggcggcgg cccagccggc cctgcacagg ttatcaacac gtttgac 47 | | | | |
| <210> | 15 | | | |
| <211> | 43 | | | |
| <212> | DNA | | | |

<223> Synthetic PCR primer used to change codon usage in recombinant cl

ones

<213> Bacillus amyloliquefaciens

| | 15 accg gtacctctga tttttgtaaa ggtctgataa gcg | 43 |
|------------------|---|----|
| <210> | 16 | |
| <211> | 44 | |
| <212> | DNA | |
| <213> | Gallus gallus | |
| | | |
| <400> | 16 ccag ceggeettte tetetetgae gaggaettea agge | 44 |
| | | |
| <210> | 17 | |
| <211> | 41 | |
| <212> | DNA | |
| <213> | Gallus gallus | |
| <400> cctcgga | 17 aacc ggtaccgaag agtcctttct ccttcttgag g | 41 |
| <210> | 18 | |
| <211> | 18 | |
| <212> | DNA | |
| <213> | Artificial sequence | |
| | | |
| <220> | | |
| <223> | Synthetic PCR primer used for library construction. | |
| <220> | | |
| <221> | misc_feature | |
| <222> | (1)(18) | |
| <223> | Synthetic PCR primer used for library construction | |

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18
tacgccaagc ttgcatgc
<210> 19
<211> 17
<212> DNA
<213> Artificial sequence
<220>
<223> Synthetic PCR primer used for library construction.
<220>
<221> misc_feature
<222> (1)..(17)
<223> Synthetic PCR primer used for library constuction
<400> 19
ctgcacctgg gccatgg
                                                                     17
<210> 20
<211> 17
<212> DNA
<213> Artificial sequence
<220>
<223> Synthetic PCR primer used for library construction.
<220>
<221> misc_feature
<222> (1)..(17)
<223> Synthetic PCR primer used for library construction
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<400> 18

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<400> 20
gattacgcca agctttg
                                                                     17
<210> 21
<211> 126
<212> DNA
<213> Erwinia chrysanthemi
<220>
<221> misc_feature
<223> n at positions 23, 24, 29, 55, 56, 81, 97, 101, and 102 can be G,
       A, T or C
<220>
<221> misc_feature
<222> (23)..(23)
<223> n at position 23 can be G, A, T or C
<220>
<221> misc_feature
<222> (24)..(24)
<223> n at position 24 can be G, A, T or C
<220>
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<222> (29)..(29)
<223> n at position 29 can be G, A, T or C
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<220>

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<221> misc_feature
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<223> n at position 55 can be G, A, T or C

<220>

<221> misc_feature

<222> (56)..(56)

<223> n at position 56 can be G, A, T or C

<220>

<221> misc_feature

<222> (81)..(81)

<223> n at position 81 can be G, A, T or C

<220>

<221> misc_feature

<222> (97)..(97)

<223> n at position 97 can be G, A, T or C

<220>

<221> misc_feature

<222> (101)..(101)

<223> n at position 101 can be G, A, T or C

<220>

<221> misc_feature

<222> (102)..(102)

<223> n at position 102 can be G, A, T or C

```
<400> 21
gattacgcca agcttgcatg cannddctnt dtcaaggaga cagtcataat garrnnbcta
                                                                     60
ttgsyaayrs yasyasyagb nttgttatta ctcsyanycv nncygdccat ggcccaggtg
                                                                    120
                                                                    126
cagctg
<210> 22
<211> 117
<212> DNA
<213> Bacteriophage M13mp18
<220>
<221> misc feature
<222> (18)..(18)
<223> Nucleotide at position 18 can be G, A, T or C.
<220>
<221> misc_feature
<222> (19)..(19)
<223> Nucleotide at position 19 can be G, A, T or C.
<220>
<221> misc_feature
<222> (20)..(20)
<223> Nucleotide at position 20 can be G, A, T or C.
<220>
<221> misc_feature
<222> (21)..(21)
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<223> Nucleotide at position 21 can be G, A, T or C.

```
<400> 22
gattacgcca agctttgnnn ncttttttww ggagattttc aacrtgaraa rattattatt
                                                                    60
csyaattsyt ttagttsyts ytttctwtgy ggyccagccg gccatggccc aggtgca
                                                                    117
<210> 23
<211> 18
<212> DNA
<213> Artificial sequence
<220>
<223> Synthetic PCR primer used for vector construction.
<400> 23
ctttatgctt ccggctcg
                                                                     18
<210> 24
<211> 17
<212> DNA
<213> Artificial sequence
<220>
<223> Synthetic PCR primer for library construction.
<220>
<221> misc_feature
<222> (1)..(17)
<223> Synthetic PCR primer for library construction
<400> 24
cggcccatt cagatcc
                                                                     17
<210> 25
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-<211> 50

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<212> DNA
<213> Artificial sequence
<220>
<223> Randomized E. chrysanthemi pelB sequence.
<220>
<221> misc_feature
<222> (1)..(50)
<223> Randomized E. chrysanthemi pelB sequence
<400> 25
                                                                     50
aagcttgcat gcaaattcta tdtcaaggag acagttataa tgaaatacct
<210> 26
<211> 50
<212> DNA
<213> Artificial sequence
<220>
      Randomized E. chrysanthemi pelB sequence.
<223>
<220>
<221> misc_feature
<222> (1)..(50)
<223> Randomized E. chrysanthemi pelB sequence
<220>
<221> misc_feature
<222> (14)..(14)
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<223> n at position 14 can be G, A, T or C.

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<220>
<221> misc_feature
<222> (15)..(15)
<223> n at position 15 can be G, A, T or C.
<220>
<221> misc_feature
<222> (20)..(20)
<223> n at position 20 can be G, A, T or C.
<220>
<221> misc_feature
<222> (45)..(45)
<223> n at position 45 can be G, A, T or C.
<220>
<221> misc_feature
<222> (46)..(46)
<223> n at position 46 can be G, A, T or C.
<400> 26
aagcttgcat gcannddctn tdtcaaggag acagtcataa tgarrnnbct
                                                                     50
<210> 27
<211> 50
<212> DNA
<213> Artificial sequence
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<220>

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<220>
<221> misc_feature
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<223> Randomized E. chrysanthemi pelB sequence
<400> 27
aagcttgcat gcagcatctc tdgcaaggag acagtcataa tgaagacgct
                                                                     50
<210> 28
<211> 50
<212> DNA
<213> Artificial sequence
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      Randomized E. chrysanthemi pelB sequence.
<220>
<221> misc_feature
<222> (1)..(50)
<223> Randomized E. chrysanthemi pelB sequence
<400> 28
aagcttgcat gcacgggctg tdtcaaggag acagtcataa tgagagggct
                                                                     50
<210> 29
<211> 50
<212> DNA
<213> Artificial sequence
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<220>

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Randomized E. chrysanthemi pelB sequence.
<223>
<220>
<221> misc feature
<222> (1)..(50)
<223> Randomized E. chrysanthemi pelB sequence
<400> 29
aagcttgcat gcaccagctc tdtcaaggag acagtcataa tgaggcggct
                                                                     50
<210> 30
<211> 55
<212> DNA
<213> Artificial sequence
<220>
<223> Randomized E. chrysanthemi pelB sequence.
<220>
<221> misc_feature
<222> (1)..(55)
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<400> 30
attectaacg geageegetg gattgttatt actegeggee cageeggeea tggee
                                                                    55
<210> 31
<211> 55
<212> DNA
<213> Artificial sequence
<220>
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<223> Randomized E. chrysanthemi pelB sequence.

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<220>
<221> misc_feature
<222> (1)..(55)
<223> Randomized E. chrysanthemi pelB sequence
<220>
<221> misc_feature
<222> (22)..(22)
<223> n at position 22 can be G, A, T or C.
<220>
<221> misc_feature
<222> (38)..(38)
<223> n at position 38 can be G, A, T or C.
<220>
<221> misc_feature
<222> (42)..(42)
<223> n at position 42 can be G, A, T or C.
<220>
<221> misc_feature
<222> (43)..(43)
<223> n at position 43 can be G, A, T or C.
<400> 31
attgsyaayr syasyasyag bnttgttatt actcsyanyc vnncygdcca tggcc
                                                                    55
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<210> 32

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<211> 55
<212> DNA
<213> Artificial sequence
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<220>
<221> misc_feature
<222> (1)..(55)
<223> Randomized E. chrysanthemi pelB sequence
<400> 32
attgcyaatg gtactgtyag gattgttatt actcccaccc ggtccgtcca tggcc
                                                                    55
<210> 33
<211> 55
<212> DNA
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<222> (1)..(55)
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<400> 33
attgcyaatg ctagtgcyag ggttgttatt actcccaatc gcgccggcca tggcc
                                                                   55
<210> 34
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<211> 54

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<212> DNA
<213> Artificial sequence
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<220>
<221> misc_feature
<222> (1)..(54)
<223> Randomized E. chrysanthemi pelB sequence
<220>
<221> misc feature
<222> (22)..(22)
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<220>
<221> misc_feature
<222> (43)..(43)
<223> n at position 43 can be G, A, T or C.
<220>
<221> misc_feature
<222> (44)..(44)
<223> n at position 44 can be G, A, T or C.
<400> 34
attggtaata gcagcagtag bnttgttagg actcgcaccc ccnncyadcc atgg
                                                                     54
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<210> 35

```
<211> 22
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<212> PRT

<213> Erwinia chrysanthemi

<400> 35

Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Ala 1 5 10 15

Ala Gln Pro Ala Met Ala 20

<210> 36

<211> 22

<212> PRT

<213> Artificial sequence

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<223> Randomized E. chrysanthemi pelB sequence.

<220>

<221> MISC_FEATURE

<222> (1)..(22)

<223> Randomized E. chrysanthemi pelB sequence

<400> 36

Met Lys Thr Leu Ala Met Val Leu Val Gly Gly Pro Pro Gly Pro Ser 1 5 10 15

Ala Gln Pro Ala Met Ala 20

<210> 37

<211> 21

<212> PRT

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Gln Pro Ala Met Ala
<210> 38
<211> 23
<212> PRT
<213> Artificial sequence
<220>
<223>
      Randomized E. chrysanthemi pelB sequence.
<220>
<221> MISC_FEATURE
<222> (1)..(23)
<223> Randomized E. chrysanthemi pelB sequence
<400> 38
Met Arg Arg Leu Val Pro Ile Thr Ala Ala Val Gly Leu Leu Ala Pro
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<210> 39
<211> 50
<212> DNA
<213> Artificial sequence
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<223> Randomized bacteriophage M13 g3 sequence.
<220>
<221> misc_feature
<222> (1)..(50)
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                                                                     50
aagctttgga cgctttttt tggagatttt caacgtgaaa aaattattat
<210> 40
<211> 50
<212> DNA
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<223> Randomized bacteriophage M13 g3 sequence.
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<221> misc_feature
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Pro Thr Gln Pro Ala Met Ala

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<220>
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<222> (1)..(50)
<223> Randomized bacteriophage M13 g3 sequence
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<221> misc_feature
<222> (10)..(10)
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<220>
<221> misc feature
<222> (11)..(11)
<223> n at position 11 is can be G, A, t or C.
<220>
<221> misc_feature
<222> (12)..(12)
<223> n at position 12 is can be G, A, t or C.
<400> 40
aagctttgnn nncttttttw wggagatttt caacrtgara arattattat
                                                                     50
<210> 41
<211> 50
<212> DNA
<213> Artificial sequence
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<220>

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<220>
<221> misc_feature
<222> (1)..(50)
<223> Randomized bacteriophage M13 g3 sequence.
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aagctttggg gcctttttt aggagatttt caacatgaga agattattat
                                                                    50
<210> 42
<211> 50
<212> DNA
<213> Artificial sequence
<220>
<223> Randomized bacteriophage M13 g3 sequence.
<220>
<221> misc_feature
<222> (1)..(50)
<223> Randomized bacteriophage M13 g3 sequence
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togcaattoo tttagttgtt cotttotatg oggoccagoo ggocatggco
                                                                    50
<210> 43
<211> 50
<212> DNA
<213> Artificial sequence
<220>
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<223> Randomized bacteriophage M13 g3 sequence.

<223> Randomized bacteriophage M13 g3 sequence.

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<220>
<221> misc_feature
<222> (1)..(50)
<223> Randomized bacteriophage M13 g3 sequence
<400> 43
                                                                     50
tcsyaattsy tttagttsyt sytttctwtg yggyccagcc ggccatggcc
<210> 44
<211> 50
<212> DNA
<213> Artificial sequence
<220>
<223> Randomized bacteriophage M13 g3 sequence.
<220>
<221> misc_feature
<222> (1)..(50)
<223> Randomized bacteriophage M13 g3 sequence
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tcctaattcc tttagttgtt gctttctatg tggtccagcc ggccatggcc
                                                                     50
<210> 45
<211> 22
<212> PRT
<213> Artificial sequence
<220>
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<223> Randomized bacteriophage M13 g3 sequence.

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<220>
<221> MISC_FEATURE
<222> (1)..(22)
<223> Randomized bacteriophage M13 g3 sequence
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Met Lys Lys Leu Leu Phe Ala Ile Pro Leu Val Val Pro Phe Tyr Ala
                                    10
Ala Gln Pro Ala Met Ala
            20
<210> 46
<211> 22
<212> PRT
<213> Artificial sequence
<220>
<223>
      Randomized bacteriophage M13 g3 sequence.
<220>
<221> MISC FEATURE
<222> (1)..(22)
<223> Randomized bacteriophage M13 g3 sequence
<400> 46
Met Arg Arg Leu Leu Leu Ala Pro Pro Val Ala Val Pro Phe Tyr Val
                                    10
                                                        15
                5
Val Gln Pro Ala Met Ala
            20
<210> 47
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<211> 18

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<212> DNA
<213> Artificial sequence
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      Synthetic oligonucleotide primer used as a substrate for Stoffel
<223>
       fragment of Thermus aquaticus DNA polymerase I.
<220>
<221> misc_feature
<222> (1)..(18)
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       agment of Thermus aquaticus DNA polymerase I
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tttcgcaaga tgtggcgt
                                                                      18
<210> 48
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<213> Artificial sequence
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      Synthetic oligonucleotide primer used as a substrate for Thermus
<223>
       aquaticus DNA polymerase I.
<220>
<221> misc feature
<222> (1)..(12)
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       s aquaticus DNA polymerase I
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gcgaagatgt gg 12

- <210> 49 <211> 30 <212> DNA <213> Artificial sequence <220> Synthetic oligonucleotide primer used as a substrate for Thermus <223> aquaticus DNA polymerase I. <220> <221> misc_feature <222> (1)..(30) <223> Synthetic oligonucleotide primer used as substrate for Thermus aq uaticus DNA polymerase I <400> 49 30 aaatacaaca ataaaacgcc acatcttgcg <210> 50 <211> 20 <212> DNA <213> Artificial sequence <220> Synthetic oligonucleotide sequence insert containing PstI restric tion site and frame shift for H102A mutant barnase fusion constru ct fused to p3 gene of phage fd-3. <220>
- tion site and frame shift for H102A mutant barnase fusion construct fused to p3 gene of phage fd-3.

<221> misc_feature

<222> (1)..(20)

Synthetic oligonucleotide sequence insert containing PstI restric

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<400> 50
ctgcaggcgg tgcggccgca
```

20

- <210> 51
- <211> 24
- <212> DNA
- <213> Artificial sequence
- <220>
- <223> Synthetic oligonucleotide used for random priming.
- <220>
- <221> misc_feature
- <222> (1)..(24)
- <223> Synthetic oligonucleotide used for random priming
- <220>
- <221> misc_feature
- <222> (19)..(19)
- <223> n at position 19 can be G, A, T or C.
- <220>
- <221> misc_feature
- <222> (20)..(20)
- <223> n at position 20 can be G, A, T or C.
- <220>
- <221> misc_feature
- <222> (21)..(21)
- <223> n at position 21 can be G, A, T or C.

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<220>
<221> misc_feature
<222> (22)..(22)
<223> n at position 22 can be G, A, T or C.
<220>
<221> misc feature
<222> (23)..(23)
<223> n at position 23 can be G, A, T or C.
<220>
<221> misc feature
<222> (24)..(24)
<223> n at position 24 can be G, A, T or C.
<400> 51
gagcctgcag agctcaggnn nnnn
                                                                      24
<210> 52
<211> 23
<212> DNA
<213> Artificial sequence
<220>
<223>
      Synthetic PCR primer used to re-amplify randomply amplified E. co
       li genomic DNA sequence.
<220>
<221> misc_feature
```

<222> (1)..(23)

<223> Synthetic PCR primer used to re-amplify randomly amplified E. col i genomic DNA sequences.

<400> 52 cgtgcgagcc tgcagagctc agg

23

<210> 53

<211> 45

<212> PRT

<213> Artificial sequence

<220>

<223> Barstar binding barnase-p3 fusion insert.

<220>

<221> MISC FEATURE

<222> (1)..(45)

<223> Barstar binding barnase-p3 fusion insert

<400> 53

Leu Gln Ser Ser Gly Asp Cys Val Ile Ser Asp Thr Cys Ile Ala Gly
1 5 10 15

Met Ala Glu Ala Ala Cys Glu Glu Lys Phe Ser Ser Gln Asn Val 20 25 30

Gly Leu Thr Ile Thr Val Thr Pro Cys Leu Ser Ser Ala 35 40 45

<210> 54

<211> 44

<212> PRT

<213> Artificial sequence

```
Barstar binding barnase-p3 fusion insert.
<220>
<221> MISC_FEATURE
<222> (1)..(44)
<223> Barstar binding barnase-p3 fusion insert
<400> 54
Leu Gln Ser Ser Gly Cys Gly Ser Ser Gly Ser Ser Ile Asn Cys Leu
                                    10
Pro Cys Gly Ala Thr Ser Arg Gly Thr Ser Pro Leu Ala Ser Gly Leu
            20
                                25
Pro Ser Ser Ala Thr Ile His Cys Leu Ser Ser Ala
<210>
      55
<211> 40
<212> PRT
<213> Artificial sequence
<220>
      Barstar binding barnase-p3 fusion insert.
<220>
<221> MISC FEATURE
<222> (1)..(40)
<223> Barstar binding barnase-p3 fusion insert
<400> 55
Leu Gln Ser Ser Gly Asp Ser Ala Gly Cys Lys Asn Met Thr Gly Gly
```

<220>

5

15

Arg Leu Tyr Ala His Thr Leu Glu Ala Ile Ile Pro Gly Phe Ala Val $20 \\ 25 \\ 30$

Ser Ala Pro Ala Cys Glu Pro Ala 35 40

<210> 56

<211> 33

<212> PRT

<213> Artificial sequence

<220>

<223> Barstar binding barnase-p3 fusion insert.

<220>

<221> MISC_FEATURE

<222> (1)..(33)

<223> Barstar binding barnase-p3 fusion insert

<400> 56

Leu Gln Ser Ser Gly Cys Val Arg Leu Lys Arg Thr Ser Val Asn His 1 5 10 15

Gln Pro Asp Ala Trp Pro Glu Pro His Leu Lys Ala Ala Cys Glu Pro 20 25 30

Ala

<210> 57

<211> 44

<212> PRT

<213> Artificial sequence

```
Barstar binding barnase-p3 fusion insert.
<220>
<221> MISC_FEATURE
<222> (1)..(44)
<223> Barstar binding barnase-p3 fusion insert
<400> 57
Leu Gln Ser Ser Gly Cys Gly Ser Ser Gly Ser Ser Ile Asn Cys Leu
                                    10
Pro Cys Gly Ala Thr Ser Arg Gly Thr Ser Pro Leu Ala Ser Gly Leu
                                25
            20
Pro Ser Ser Ala Thr Val Gln Cys Leu Ser Ser Ala
                            40
        35
<210>
      58
<211> 41
<212> PRT
<213> Artificial sequence
<220>
      Barstar binding barnase-p3 fusion insert.
<220>
<221> MISC FEATURE
<222>
     (1)..(41)
<223> Barstar binding barnase-p3 fusion insert
<400> 58
Leu Gln Ser Ser Gly Lys Ile Val Gln Ala Gly Ala Asn Ile Gln Asp
```

<220>

15

5

Gly Cys Ile Met His Gly Tyr Cys Asp Thr Asp Thr Ile Val Gly Glu 20 25 30

Asn Gly His Ile Gly Leu Ser Ser Ala 35 40

<210> 59

<211> 45

<212> PRT

<213> Artificial sequence

<220>

<223> Barstar binding barnase-p3 fusion insert.

<220>

<221> MISC_FEATURE

<222> (1)..(45)

<223> Barstar binding barnase-p3 fusion insert

<400> 59

Leu Gln Ser Ser Gly Val Cys Val Ile Ser Asp Thr Cys Ile Ala Gly
1 5 10 15

Thr Ala Glu Ala Ala Cys Glu Glu Lys Phe Ser Ser Gln Asn Val 20 25 30

Gly His Thr Ile Thr Glu Thr Pro Cys Leu Ser Ser Ala 35 40 45

<210> 60

<211> 44

<212> PRT

<213> Artificial sequence

```
<220>
       Barstar binding barnase-p3 fusion insert.
<223>
<220>
<221> MISC FEATURE
<222> (1)..(44)
<223> Barstar binding barnase-p3 fusion insert
<400> 60
Leu Gln Ser Ser Gly Cys Gly Ser Ser Gly Ser Ser Ile Asn Cys Leu
                                    10
Pro Cys Gly Ala Thr Ser Arg Gly Thr Ser Pro Leu Ala Ser Gly Leu
Pro Ser Ser Ala Thr Ile Gln Cys Leu Ser Ser Ala
        35
<210>
      61
<211> 53
<212> PRT
<213> Artificial sequence
<220>
       Barstar binding barnase-p3 fusion insert.
<220>
<221> MISC FEATURE
<222> (1)..(53)
<223> Barstar binding barnase-p3 fusion insert
<400> 61
Leu Gln Ser Ser Gly Gln Asp Ser Gln Arg Glu His Ala Ser His Thr
```

10

15

5

Ala Glu Asp Asp Cys Glu Asp Gln Thr Arg Ile His Gln His Ile Arg
20 25 30

Glu Val Asp Phe Val Asp Thr Pro Gln Glu Val Asp Asp Cys Arg Ala 35 40 45

Ala Leu Ser Ser Ala 50

<210> 62

<211> 33

<212> PRT

<213> Artificial sequence

<220>

<223> Barstar binding barnase-p3 fusion insert.

<220>

<221> MISC_FEATURE

<222> (1)..(33)

<223> Barstar binding barnase-p3 fusion insert

<400> 62

Leu Gln Ser Ser Gly Cys Val Arg Leu Lys Arg Thr Ser Val Asn His 1 5 10 15

Gln Pro Asp Ala Trp Pro Glu Pro His Leu Lys Ala Ala Cys Glu Pro 20 25 30

Ala

<210> 63

<211> 9

<212> PRT

```
<213> Artificial sequence
<220>
       Barstar binding barnase-p3 fusion insert.
<223>
<220>
<221> MISC_FEATURE
<222> (1)..(9)
<223> Barstar binding barnase-p3 fusion insert
<400> 63
Leu Gln Ser Ser Gly Val Arg Pro Ala
<210> 64
<211> 44
<212> PRT
<213> Artificial sequence
<220>
<223> Barstar binding barnase-p3 fusion insert.
<220>
<221> MISC FEATURE
<222> (1)..(44)
<223> Barstar binding barnase-p3 fusion insert
<400> 64
Leu Gln Ser Ser Gly Cys Gly Ser Ser Gly Ser Ser Ile Asn Cys Leu
               5
                                    10
                                                        15
Pro Cys Gly Ala Thr Ser Arg Gly Thr Ser Pro Leu Ala Ser Gly Leu
```

20

```
Pro Ser Ser Ala Thr Ile Gln Cys Leu Ser Ser Ala
        35
                            40
<210> 65
<211> 30
<212> PRT
<213> Artificial sequence
<220>
<223>
     Barstar binding barnase-p3 fusion insert.
<220>
<221> MISC FEATURE
<222> (1)..(30)
<223> Barstar binding barnase-p3 fusion insert
<400> 65
Leu Gln Ser Ser Gly Thr Glu Val Asp Arg Gly Asn Gln Gln His Asp
                                    10
Thr Asn Asp Arg Asp Phe Thr His Thr Pro Leu Ser Ser Ala
           20
                                25
<210>
      66
<211>
     36
<212> PRT
<213> Artificial sequence
<220>
<223> Barstar binding barnase-p3 fusion insert.
<220>
<221> MISC FEATURE
```

```
<222> (1)..(36)
```

<223> Barstar binding barnase-p3 fusion insert

<400> 66

Leu Gln Ser Ser Gly Val Ala Gln Gly Ser Ser Ala Ser Val Asp Val
1 5 10 15

Thr Ala Thr Asn Ala Val Leu Ser Ala Asp Ser Leu Ser Leu Gly Gly 20 25 30

Gly Glu Pro Ala 35

<210> 67

<211> 19

<212> PRT

<213> Artificial sequence

<220>

<223> Barstar binding barnase-p3 fusion insert.

<220>

<221> MISC_FEATURE

<222> (1)..(19)

<223> Barstar binding barnase-p3 fusion insert

<400> 67

Leu Gln Ser Ser Gly Gly Ala Val Ala Val Thr Pro Gly Pro Val Leu 1 5 10 15

Ser Ser Ala

<210> 68

<211> 18

```
<212> PRT
<213> Artificial sequence
<220>
<223> Barstar binding barnase-p3 fusion insert.
<220>
<221> MISC_FEATURE
<222> (1)..(18)
<223> Barstar binding barnase-p3 fusion insert
<400> 68
Leu Gln Ser Ser Gly His Cys Arg Gly Lys Pro Val Leu Cys Thr His
Thr Ala
<210> 69
<211> 9
<212> PRT
<213> Artificial sequence
<220>
<223> Barstar binding barnase-p3 fusion insert.
<220>
<221> MISC_FEATURE
<222> (1)..(9)
<223> Barstar binding barnase-p3 fusion insert
```

<400> 69

```
Leu Gln Ser Ser Gly Val Arg Pro Ala
                5
<210> 70
<211> 36
<212> PRT
<213> Artificial sequence
<220>
<223> Barstar binding barnase-p3 fusion insert.
<220>
<221> MISC_FEATURE
<222> (1)..(36)
<223> Barstar binding barnase-p3 fusion insert
<400> 70
Leu Gln Ser Ser Gly Glu Pro Ala Pro Ala His Glu Ala Lys Pro Thr
               5
                                                       15
Glu Ala Pro Val Ala Lys Ala Glu Ala Lys Pro Glu Thr Pro Ala His
Leu Ser Ser Ala
       35
<210>
      71
<211> 33
<212> PRT
<213> Artificial sequence
<220>
<223> Barstar binding barnase-p3 fusion insert.
```

<220>

```
<221> MISC_FEATURE
<222> (1)..(33)
<223> Barstar binding barnase-p3 fusion insert
<400> 71
Leu Gln Ser Ser Gly Cys Val Arg Leu Lys Arg Thr Ser Val Asn His
Gln Pro Asp Ala Trp Pro Glu Pro His Leu Lys Ala Ala Cys Glu Pro
            20
Ala
<210> 72
<211> 36
<212> PRT
<213> Artificial sequence
<220>
<223>
      Barstar binding barnase-p3 fusion insert.
<220>
<221> MISC FEATURE
<222> (1)..(36)
<223> Barstar binding barnase-p3 fusion insert
<400> 72
```

Asp Ser Ile Gly Ala Tyr Leu Phe Val Asp Met Ala His Val Ala Ala 20 25 30

Leu Gln Ser Ser Gly Val Val Asp Trp Ala Lys Met Arg Glu Ile Ala

10

```
Leu Ser Ser Ala
        35
<210> 73
<211> 117
<212> DNA
<213> Artificial sequence
<220>
<223> Vector pK1 polylinker sequence.
<220>
<221> misc feature
<222> (1)..(117)
<223> Vector pK1 polylinker sequence
<400> 73
aatgctggcg gcggcccagc cggcctttct gaggggtcga ctatagaagg acgaggggcc
                                                                    60
cacgaaggag gtggggtacc cggttccgag ggtggttccg gttccggtga ttttgat
                                                                    117
<210> 74
<211> 39
<212> PRT
<213> Artificial sequence
<220>
<223> Polypeptide encoded by pK1 vector polylinker sequence.
<220>
<221> MISC_FEATURE
<222> (1)..(39)
<223> Polypeptide encoded by pK1 vector polylinker sequence
```

```
<400> 74
Asn Ala Gly Gly Gly Pro Ala Gly Leu Ser Glu Gly Ser Thr Ile Glu
Gly Arg Gly Ala His Glu Gly Gly Gly Val Pro Gly Ser Glu Gly Gly
                                25
            20
Ser Gly Ser Gly Asp Phe Asp
<210>
      75
<211> 117
<212> DNA
<213> Artificial sequence
<220>
      Vector pK2 polylinker sequence.
<223>
<220>
<221> misc_feature
<222> (1)..(117)
<223> vector pK2 polylinker sequence
<400> 75
aatgctggcg gcggcccagc cggcctttct gaggggtcga ctatagaagg acgagggccc
                                                                     60
acgaagcagc tggggtaccg gttccgaggg tggttccggt tccggtgatt ttgatta
                                                                    117
<210> 76
<211> 39
<212> PRT
<213> Artificial sequence
```

47

<220>

```
<223> Polypeptide sequence encoded by vector pK2 polylinker region.
```

<220>

<221> MISC_FEATURE

<222> (1)..(39)

<223> Polypeptide sequence encoded by vector pK2 polylinker region.

<220>

<221> MISC FEATURE

<222> (38)..(38)

<223> X represents a TGA stop codon

<220>

<221> MISC FEATURE

<222> (36)..(36)

<223> X represents a stop codon (TGA)

<400> 76

Asn Ala Gly Gly Gly Pro Ala Gly Leu Ser Glu Gly Ser Thr Ile Glu 1 5 10 15

Gly Arg Gly Pro Thr Lys Gln Leu Gly Tyr Arg Phe Arg Gly Trp Phe 20 25 30

Arg Phe Arg Xaa Phe Xaa Leu 35

<210> 77

<211> 35

<212> DNA

<213> Artificial sequence

<220>

```
<223> Sequence of the junction region between Barnase and p3 in recombi
       nant fusion vector fd-3.
<220>
<221> misc_feature
<222> (1)..(35)
<223> Sequence of the junction region between Barnase and p3 in recombi
       nant fusion vector fd-3.
<400> 77
                                                                     35
atcagactgc aggcggtgcg gccgcagaaa ctgtt
<210> 78
<211> 11
<212> PRT
<213> Artificial sequence
<220>
<223> Amino acid sequence about the junction of Barnase and p3 coding r
       egions of recombinant fusion vector fd-3.
<400> 78
Ile Arg Leu Gln Ala Ala Ala Glu Thr Val
<210> 79
<211> 4
<212> PRT
<213> Artificial sequence
<220>
<223> Factor Xa protease cleavage sequence.
<220>
```

<221> MISC FEATURE

```
<222> (1)..(1)
```

<223> X can be either Ile or Leu.

<220>

<221> MISC_FEATURE

<222> (1)..(4)

<223> Factor Xa proteolytic cleavage site.

<400> 79

Xaa Glu Gly Arg

<210> 80

<211> 6

<212> PRT

<213> Artificial sequence

<220>

<223> Artificial linker peptide.

<400> 80

Ala Gly Gly Ala Ala Ala